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Project Summary

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Restoring Hazardous Spill-Damaged Areas: Technique Identification/Assessment

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This study identifies and assesses methods for accelerating the restoration of lands damaged by spills of hazardous materials. The first phase of the study involved a literature review to determine what response methods had been used in the past to clean up spills on land and to identify other techniques that could be developed for detoxification of hazardous-spill-damaged lands.

In the second phase of the study, four primarily biological techniques for accelerating the restoration of spill-damaged lands were evaluated in the laboratory:

Technique	Chemical
1. Enhancement of microbial degradation by indigenous organisms	Chlorobenzene, Ethion
2. Addition of mixed microorganisms from primary sewage effluent	Formaldehyde, aniline
3. Addition of adapted/mutant microbial cultures	Dinitrophenol, chlordane
4. Selective absorption by harvestable plants	Lead nitrate, cadmium nitrate

The accelerated removal of one or both chemicals was observed in techniques 2, 3, and 4. The effects of the spilled chemical on the soil chemistry and microorganisms were also monitored.

During the third phase, a plan for field testing of techniques 2 and 4 was designed. Recommended land restoration methods for spills of the 271 hazardous chemicals listed in the Federal Register (1978) were compiled during the fourth phase.

This Project Summary was developed by EPA's Municipal Environmental Research Laboratory, Cincinnati, OH, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

This study focused on the identification and development of biological techniques that have the potential for restoration of spill-damaged lands and a laboratory assessment of the feasibility of these techniques. A literature search was conducted to determine the current biological, chemical, and physical techniques used to treat spill-damaged land. Viable biological techniques were then identified, and four were selected for laboratory evaluation.

The first technique examined was the enhancement of microbial degradation by indigenous organisms. This technique was tested for several reasons. First, the microorganisms that have

survived the initial shock from the spilled chemical are relatively resistant to the chemical. If the surviving microbial population can be increased rapidly with the addition of nutrients, they may then be able to degrade the chemical. Also, the organisms used are from the spill area and do not have to be specially cultured.

The second technique (T-1) involved the use of microorganisms in primary sewage effluent to degrade a spilled chemical. Primary sewage effluent contains a variety of microorganisms, is usually high in nutrients, is cheap, and is readily available. Primary sewage effluent can be used to replenish the microbial population in the spill area. If nutrients are supplied, the microorganisms from the primary sewage effluent can degrade the spilled chemical.

The third technique (T-2) was the addition of adapted/mutant microbial cultures. Soil or primary sewage effluent microorganisms were cultured in nutrient medium containing the chemical to be degraded. The chemical was the sole carbon source for the organisms. After several subcultures, a culture was developed that could utilize the chemical of concern. This culture, with nutrients, could then be applied to the soil to degrade the spilled chemical.

The fourth technique evaluated was the uptake of heavy metals by harvestable plants. Plants were selected for use based on data from the literature search and preliminary tests. For the heavy-metal-contaminated soil, conditions for uptake by the plants were optimized by adjusting the pH and adding disodium ethylenediaminetetra acetic acid, a chelating agent.

A plan for field testing of the two most promising techniques from the laboratory tests was then developed. The plan included the chemicals to be spilled, restoration treatments to be applied, and sampling techniques.

Finally, recommended land restoration methods were developed for the hazardous chemicals under consideration. The chemicals were environmentally classified, and restoration techniques were proposed for treatment.

Experimental Methodology

Two 2.7- x 5.8-m greenhouses were used in the study. Each greenhouse contained nine enclosed environmental chambers that were constructed in groups of three, with each group supported by a wood frame. A diagram of three environmental chambers is shown in Figure 1.

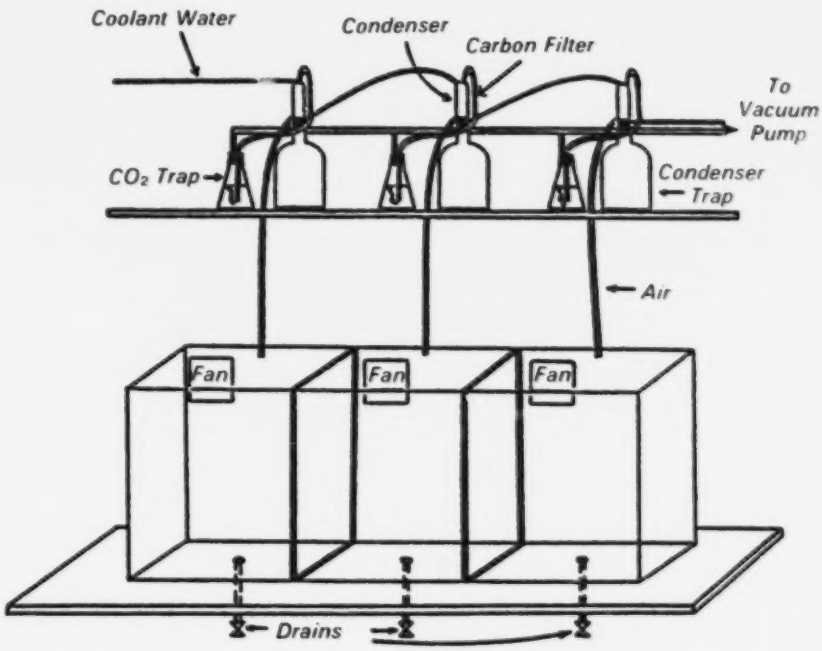


Figure 1. Set of three environmental chambers.

A steady flow of fresh air through the environmental chambers was induced by connecting the air exit line from the chamber to a vacuum system. Fresh outside air was admitted into each environmental chamber, recirculated within the chambers by means of a small fan, and then passed through a water-cooled condenser, a carbon filter, a sodium hydroxide bubbler, and a vacuum pump to the outside. The air system permitted all airborne chemicals to be collected for analysis.

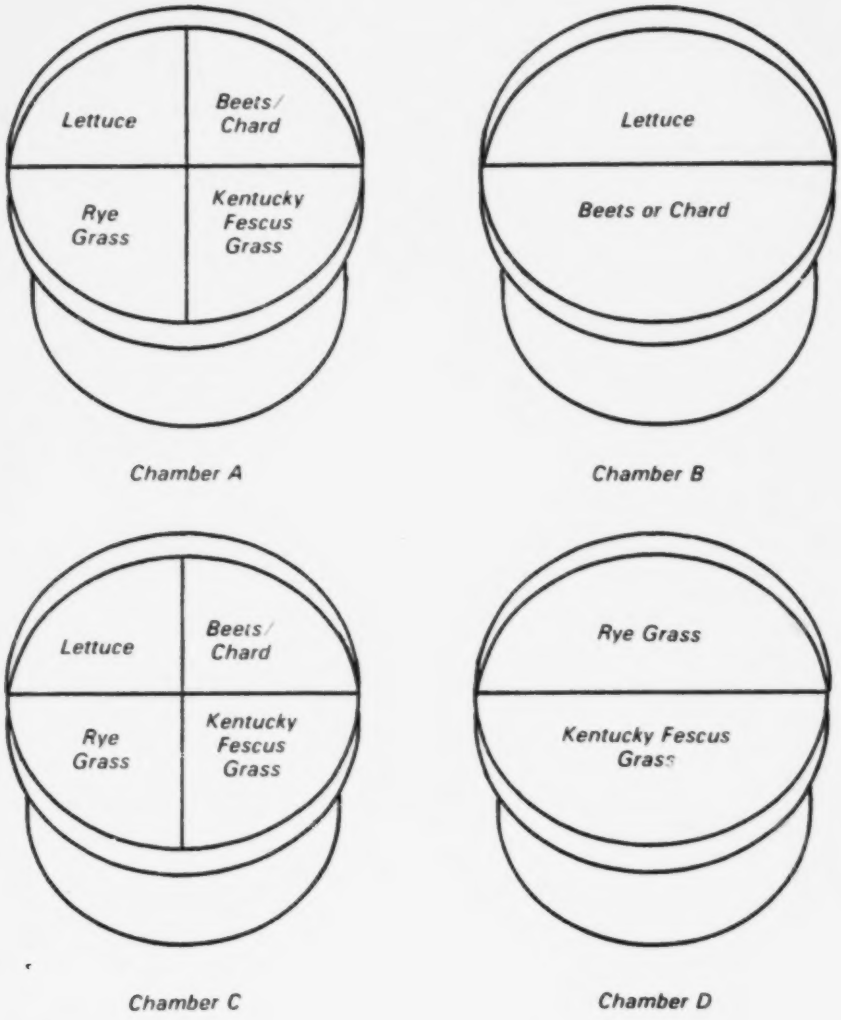
A liquid drainage hole was drilled in the glass bottom of each environmental chamber. Any liquid collected in the bottom of the chambers could be drained and analyzed.

In the bottom of each chamber were placed 2.5 cm of sand, then 5 cm of gravel, and another 2 cm of sand. Approximately 30 to 45 cm of soil were then placed in the chambers (high-organic soil in three chambers, sandy-loam in three chambers, and clay in the remaining three chambers). Once the chambers were filled with soil and packed down, the soils were seeded with perennial rye grass and allowed to equilibrate for 3 weeks.

Two liters of the liquid organic chemical under study were evenly sprinkled over each of the nine chambers. 2,4-Dinitrophenol, a solid at room temperature, was applied at a density of 500 g/chamber. After application of the chemical, the chambers were closed, and the air circulation system was put into operation. Core samples were taken 24 and 48 hours after chemical addition and approximately every week thereafter.

One set of three chambers (organic, sandy, and clay soils) was used as a control and received no treatment. The other two sets received experimental treatments falling within the definition of the three techniques outlined above.

Planters 45 cm in diameter and 45 cm high were used to study the uptake of heavy metals by harvestable plants. A diagram of the planters and the experimental treatments are presented in Figure 2. Twelve planters were used for each metal. Three soils were used in the test—organic, sandy-loam, and clay—and four planters were used for each soil. For each soil, one planter acted as a control and the other three received the heavy metal contamination. Three of the four planters for each soil type were sprinkled with 250 ml of 0.025 molar Cd



Chamber Treatments

A (the control): no metal, pH adjusted.
B and D: metal added, pH adjusted.
C: metal added, pH adjusted, chelating agent added.

Figure 2. *Experimental design for plant uptake studies.*

$(NO_3)_2$, and another set of planters was doused with 250 ml of 0.025 molar Pb $(NO_3)_2$.

The soil samples from each chamber that had been doused with organic compounds were analyzed for soil pH, percent moisture, percent organic matter, nitrate, soil bacteria, soil fungi, and soil ^{14}C activity (except for 2,4-dinitrophenol, which did not contain ^{14}C-labeled molecules). The heavy metal tests also included an analysis of the soil and of sampled plant tissue for Cd or Pb.

Results and Discussion

Monochlorobenzene

Monochlorobenzene was somewhat toxic to soil bacteria and was highly toxic to grass. Twenty-four hours after the spill, the grass had completely turned brown. Monochlorobenzene was rapidly lost from the soil, as evidenced by the high concentration of it found in the carbon filters and traps.

No evidence of microbial degradation of monochlorobenzene was observed during the tests. No additional compounds were seen in the GC traces, and no $^{14}CO_2$ was found in the sodium hydroxide bubblers.

The monochlorobenzene provided a good example of the movement in soils of a volatile compound that is low in polarity and low in water solubility. The monochlorobenzene evaporated from the soils tested within 28 days after the spills. The treatment methods applied to the soils were relatively ineffective because they involved wetting the soil surface. The wet soil reduced the evaporation rate of the monochlorobenzene in the treated chambers. The control chambers had uninhibited evaporation of the monochlorobenzene from the soil and a faster recovery of normal soil fauna.

Ethion

Ethion did not have an immediate effect on the grass, which remained healthy in all chambers for about 1 month after the spill and then slowly wilted and turned brown. During the first 5 months after the spill, the Ethion* levels in the soils gradually decreased. The initial treatments, with nutrients (T-1 chambers) or nutrients with aeration (T-2 chambers) were not effective in significantly increasing the degradation rate of Ethion by indigenous microorganisms. Adjustment of soil pH with lime addition on day 44, 46, and 53 did not improve the Ethion degradation rate.

The microbial populations were sufficiently high in the treated chambers, and the microbes appeared to be capable of living in the presence of Ethion. But, either the organisms were incapable of degrading the Ethion, or the Ethion was unavailable to the organisms. Two methods were tried to improve the water solubility of Ethion and thus its availability to the microbial populations: (a) the addition of a surfactant (Tween 80), and (b) the addition of ethanol. These treatments were applied to the T-1 and T-2

*Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

chambers on day 95 and day 122, respectively, but they did not improve the Ethion degradation rate by microorganisms.

On day 210, day 219, and day 220, the T-2 chambers were each treated with a total of 10 liters of a 50:50 ethanol-water solution containing 6.8% sodium hydroxide to determine if the Ethion could be hydrolyzed in the soil. The pH of the top layer of soil was raised to 10 to 12 by this treatment. Because of the high pH, microbial levels dropped drastically. In the organic soil, the hydrolysis treatment reduced the Ethion levels from 55,000 ppm to less than 4,000 ppm. Ethion levels in the top layer of the sandy soil decreased from approximately 60,000 ppm to less than 10,000 ppm. The Ethion levels in the clay soil were not substantially affected by the treatment.

Following the ethanol/sodium hydroxide treatment, a number of tentative degradation products were observed by gas chromatography/mass spectroscopy. None of the observed peaks could be matched to entries in the NIH/EPA Mass Spectral Search System (MSSS). Tentative molecular formulae and possible structural assignments for several of the peaks are presented. Confirmation of identity without isolation of sufficient material for nuclear magnetic resonance (NMR) and infrared spectra is not possible.

The ^{14}C radiation levels in all soils were variable but indicated a loss of activity with time. After the ethanol/sodium hydroxide treatment, the radiation levels did not decrease significantly, indicating that the S-CH_2-S segment of the Ethion molecule (the ^{14}C-labeled site) was still present in the soil.

Assuming a first-order decay of Ethion in the three soil types, the half-lives of Ethion were calculated from the ^{14}C measurements and from chemical analysis (Table 1).

Table 1. *Ethion Half-Lives*

	Time (days)	
Soil Type	Chemical analysis	^{14}C
Organic	157	162
Sandy	200	117
Clay	123	159

Table 2. *Rate of Formaldehyde Loss*

	Chemical Analysis (ppm/day)			^{14}C Activity (cpm/day)		
Chambers	Organic	Sandy	Clay	Organic	Sandy	Clay
Control	145	82	48	2.3	1.3	0.7
T-1	110	90	64	4.5	1.1	1.0
T-2	122	51	72	1.8	0.5	1.0

Formaldehyde

The spill of formaldehyde was toxic to both the soil microorganisms and the plant life. The grass was dead by the end of the second day after the spill. On days 2 and 25 after the formaldehyde spill, one set of soil chambers was treated with primary sewage effluent (T-1) and another set was treated with primary sewage effluent cultured with 5000 ppm formaldehyde (T-2). In the organic soils, formaldehyde concentrations in the treated and control chambers were essentially the same for the first 25 days. The second microbial treatment applied on day 25 was again ineffective in accelerating the removal of formaldehyde from the soil. The radiometric data generally confirm the formaldehyde analyses.

The microbial populations in both the control and treated chambers gained viability when formaldehyde levels decreased to approximately 1000 ppm. Once this occurred, the formaldehyde levels in the soil decreased rapidly. When the formaldehyde is reduced to tolerable levels, the organic soil provides adequate nutrients for microbial metabolism.

The sandy soil upper layers exhibited only slight differences in formaldehyde levels between the treated and control chambers over the initial 25 days. After the second treatment was applied on the 25th day, however, both T-1 and T-2 chamber soils had lower formaldehyde levels than the control. The T-1 chambers had the most rapid loss of formaldehyde. The middle sandy soil layers of the three chambers had approximately the same formaldehyde concentrations for the first 20 days, but they continued to decline from day 20 to 66 in the T-1 and T-2 chambers and the concentration in the control chamber remained effectively stable. The treated and control chambers had no significant differences in formaldehyde concentrations at the lower level. The concentrations gradually declined throughout the study.

The formaldehyde levels in the upper layer of all the clay soil chambers were approximately the same during the first 25 days after the spill. The second treatment of microorganisms (day 25) increased the rate of depletion of the formaldehyde in the treated chambers. The formaldehyde concentration disappeared from the T-2 chambers at a faster rate than from the T-1 chambers. In the control chamber, however, the rate of disappearance of formaldehyde remained constant. By the 47th day, both the T-1 and T-2 chambers had upper layer formaldehyde concentrations of less than 100 ppm, whereas the control chamber contained more than 1000 ppm of formaldehyde in the soil.

The middle soil layer in the clay chambers had initial formaldehyde concentrations between 500 and 1000 ppm. The T-2 chamber middle layer reached 0 ppm of formaldehyde on day 52. The lower clay soil layers showed no significant trends in formaldehyde concentration that could be attributed to the treatments used. All lower clay layers had formaldehyde concentrations of less than 30 ppm on the 46th day.

The rate of formaldehyde loss determined from regression lines fitted to formaldehyde concentration versus time measurements in the upper soil layers and to the ^{14}C counts per minute (cpm) data are given in Table 2.

The higher rate of formaldehyde removal from the sandy T-1 and clay T-2 chambers correlates with the loss of ^{14}C activity in the chambers.

Aniline

The aniline spill was toxic to soil microbes and plants. The grass in the chamber was dead by the end of the fourth day after the spill.

The initial treatments with primary sewage effluent (T-1) on days 2 and 42 or with 15% hydrogen peroxide solution and primary sewage effluent (T-2) on days 2 and 12 were relatively ineffective in reducing aniline levels in the organic soil. On day 96, however, the T-1 chambers were treated with a mixed

culture of microorganisms and a nutrient salt solution that resulted in aniline levels significantly lower than in the control chamber by day 113. The addition of nutrient salt and yeast extract solutions to the T-1 chambers on days 126 and 154 continued to reduce the aniline levels. By day 160, aniline concentration in the T-1 chambers was less than 100 ppm. The treatments applied to the T-2 chambers were not effective in reducing the aniline concentrations in the soil.

The hydrogen peroxide treatments reduced the aniline concentrations in the T-2 sandy chamber by 20% to 40%. This T-2 chamber had the lowest aniline concentrations for the remainder of the experiment. The initial addition of primary sewage effluent to the sandy soil T-1 chamber was ineffective; but after the addition of the mixed microbial culture and the nutrient salts on day 96, the aniline level decreased by 6000 to 8000 ppm. Another decrease in the soil aniline concentration in this T-1 chamber was observed after the nutrient salts and yeast extract were added on day 154.

None of the treatments were effective in accelerating the removal of aniline from the clay soil. Aniline levels in all three chambers decreased at a similar rate.

The half-life data for aniline in the organic and sandy soils are presented in Table 3. The half-life was calculated assuming a first-order decay. For the organic soil, the aniline concentration in the control chamber on day 12 was used as the initial value for the calculations. The half-life value of aniline in the T-1 chamber was about half those obtained in the control and T-2 chambers.

The half-lives for the sandy soil chambers were separated in parts. In the first part of the experiment (days 7 to 91), the T-2 chamber (which received hydrogen peroxide) had the shortest half-life. In the second part of the experiment (days 91-166), the T-1 chamber had the shortest half-life. The T-1 chamber received the adapted mixed microbial culture and nutrient salts during this period.

Chlordane

The chlordane spill was not significantly toxic to soil microbes, but the grass was completely dead within 48 hours after the spill. On days 2, 8, and 28, adapted microbial culture and nutrient salts were added to both sets of chambers (T-1 and T-2), and the T-2 chambers were also treated with lime.

In general, there was no loss of chlordane from the clay or the organic soils. The organic T-1 soil showed a slight decline in chlordane; however, the data are too scattered to draw any definite conclusions. In all sandy soil upper layers, chlordane concentrations decreased. The control and treated chambers appeared to lose chlordane at a similar rate. The ^{14}C radiation levels in the sandy soils also show a gradual decrease in activity. No chlordane was found in the traps or carbon filters or in any of the chambers. The ^{14}C levels in the bubblers from the sandy chambers were below the detection limits.

From the data, it appears that chlordane is persistent in the soils with the possible exception of the sandy soils. Whether the apparent decrease in chlordane levels in sandy soil resulted from degradation or volatilization is not known. No degradation products were found in the GC traces, but column conditions, or extraction conditions, or both may not have been favorable for their detection.

2,4-Dinitrophenol

This chemical killed the grass cover in the chambers within 5 days after the spill and depressed the soil microbial populations. On days 2 and 11, adapted microbial culture, nutrient salts, and yeast extract were added to one set of chambers. In addition, these chambers were treated with lime on day 2.

The 2,4-dinitrophenol concentrations in the upper layer of the treated and control sandy soil chambers were similar throughout the experiment. The concentration was observed to decrease from near 4000 ppm at day 1 to 1200 to 1400 ppm at day 62. The treatments were not effective in increasing the 2,4-dinitrophenol removal rate from the sandy soil.

The treated clay soil chamber appeared to have an initial 50% loss of 2,4-dinitrophenol concentration in the upper layer during the first 12 days. After day 12, the upper layer 2,4-dinitrophenol concentrations were relatively stable between 500 and 1000 ppm. The 2,4-dinitrophenol concentration in the upper layer clay soil control chamber was near 2000 ppm on day 12, and it gradually decreased to 1000 ppm by day 72. After the initial treatment of the microorganisms, the 2,4-dinitrophenol concentrations in the treated clay soil chamber were reduced by at least 1000 ppm. But further reductions in the 2,4-dinitrophenol levels did not occur.

Heavy Metal Bioaccumulation

The experimental design for the heavy metal tests is presented in Figure 2. The plants grown in the chelated soils had higher metal contents. Chelating agents increased the plant uptake of metals by solubilizing them and increasing their diffusion to root surfaces. The disodium EDTA was added to the soil only once in the early part of the experiment. This chemical is biodegradable and was probably removed from the soil during the course of the experiments. Repeated applications of EDTA or other chelating agents would assuredly increase the removal rate of the metals from the soil. For cadmium, EDTA significantly increased the uptake of the metal by the vegetation. The results were not as dramatic for lead.

The reduction of cadmium concentrations in the chelated soils during the experiment was due to the uptake of cadmium by the plants and the leaching of the metal from the soil. The cadmium concentrations in the chelated sandy soil chamber decreased by about 50%. The grasses sampled from the chamber during the first 30 days accounted for 1% to 2% of the metal loss. If the grass had been planted in the entire chamber and complete harvesting had been conducted, the amount of metal removed would have been greater. But at least several harvests would need to be conducted before significant losses of cadmium from the soil would occur.

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Table 3. *Half-Life of Aniline in Organic and Sandy Soils*

	Organic soil	*Sandy soil*	
Chamber	*Days 12 to 166*	*Days 7 to 91*	*Days 91 to 166*
Control	*44*	*39*	*45*
T-1	*19*	*63*	*24*
T-2	*58*	*28*	*49*

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***John Brugger** is the EPA Project Officer (see below).*
The complete report, entitled "Restoring Hazardous Spill-Damaged Areas: Technique Identification/Assessment," (Order No. PB 82-103 870; Cost: $26.00, subject to change) will be available only from:
National Technical Information Service
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